Standard Operating Procedure for the Determination of Chlorophyll A

1.0 Location

Chlorophyll A determinations are performed in the spectroscopy laboratory, room 309.

2.0 Purpose

The purpose of this procedure is to determine the concentration of photo synthetic pigments to estimate phytoplankton biomass.

3.0 Scope

This procedure can be used to determine the concentration of Chlorophyll A and/or B in marine and fresh water samples.

4.0 Reference

Standard Methods for the Examination of Water and Wastewater, 17th Edition, 1998, Method 10200 H, pp 10-18 to 10-25.

5.0 Sample Handling

5.1 Samples should be filtered as soon as possible.

5.2 Samples should be filtered through glass fiber filters, optimally using an 1000ml sample size. Alternatively use a maximum of two filters to obtain the closest to 1000 ml sample size as possible.

5.3 The filtered concentrate should be placed in a plastic petri dish and covered with foil.

5.4 Samples should be cooled or frozen as soon as possible.

5.5 Samples must be analyzed within three weeks after collection and may be stored frozen until then.

5.6 Samples from acidic waters must be processed immediately.

6.0 Apparatus and Materials

6.1 Equipment

6.1.1 Sample Homogenizer (VirTis Homogenizer located in Petroleum Lab).

6.1.2 Screw cap polypropylene tubes, 2.0 *u*m filter assembly, and plunger. Disposable 0.45 *u*m filter and syringe.

6.1.3 Centrifuge tubes, 15 ml graduated, with screw cap.

6.1.4 Spectrophotometer, narrow band width (0.5-2 nm).

6.1.5 Squeeze bottle for acetone, 10 ml tip volume dispenser for aqueous acetone.

6.1.6 Cuvettes, 1 cm path length.

6.1.7 3 ml volumetric pipettes, 100 *u*l pipetter and tips.

6.1.8 Timer able to count down 1 minute.

6.2 Reagents

6.2.1 Saturated magnesium carbonate solution: Add 1.0 gram finely powdered Mg CO_3 to 100 ml de ionized water.

6.2.2 Acetone, reagent grade BP 56ºC.

6.2.3 Aqueous acetone solution: mix 90 parts acetone (reagent grade BP 56ºC) with 10 parts saturated magnesium carbonate solution.

6.2.4 Hydrochloric acid 0.1 N.

7.0 Procedures

7.1 Instrument Operation

7.1.1 Use Beckman DU-7 spectrophotometer in Petroleum lab..

7.1.2 Switch on power to the instrument, wait for self test to complete.

7.1.3 Press the **on idle** key, wait for self test to complete.

7.1.4 Press **UV** and **VIS** key to turn on the light sources (listen for the

solenoids to switch), wait for UV indicator to stop blinking.

7.1.5 Press the **Scan** key. Set the following parameters. Use arrow keys to move and **Sel** to change selections. For numbers, type number and press enter.

7.1.5.1	Function	(Abs)
7.1.5.2	Starting λ	760
7.1.5.3	Ending λ	600
7.1.5.4	Speed	(120)
7.1.5.5	Calculation	(Peak Pick)
7.1.5.6	Upper limit	2
7.1.5.7	Lower limit	0

7.1.6 Press **Multi λ** key and enter the six required wavelengths ....$665_{a,}$ 750_{a}, 664_{b}, 750_{b}, 647_{b}, 630_{b}.

7.1.7 To calibrate instrument. Insert curvette containing 90% acetone blank. Press the **Start** key and wait for the cycle to complete. After calibration, upon pressing the **Run** key the optical density (OD) of the blank should be as close to zero as possible. Recalibration may be required.

7.1.8 When prompted, insert sample in same cuvette. Press Run and wait.

7.2 Extraction Procedure

7.2.1 Obtain samples and allow them to thaw if needed. Prepare chlorophyll work sheet, located in Microsoft Excel - Chlorx.xls, edit sample numbers and enter appropriate data.

7.2.2 Fold filter carefully using small spatula, avoid touching with fingers. Place filter into polypropylene tube.

7.2.3 Using the dispenser, add 10 ml of 90% aqueous acetone solution and

macerate filter with VirTis on low speed until filter is completely disintegrated, about 1 minute.

7.2.4 Place screw caps on tubes and steep samples at least 2 hours at 4° C. Can Be left overnight.

7.2.5 Conduct work with chlorophyll extract in subdued light as much as possible. Keep tubes of extract covered with foil at all times.

7.2.6 Clarify extract by forcing 2.0 um filtering device with plunger through the acetone/filter slurry. Pour off extract into a disposable syringe with a 0.45 um filter attached. Collect into a 15 ml screw cap centrifuge tube.

7.3 Spectrophotometric Procedure

7.3.1 Proceed according to section 7.1 for instrument operation.

7.3.2 Do background first, using 90% aqueous acetone.

7.3.3 Rinse cuvette with acetone prior to use. Allow to dry between samples.

7.3.4 Pipet 3 ml sample into cuvette.

7.3.5 When prompted, insert sample in curvette. Press the **Run** key and wait for the scan to complete. The OD 664_b, OD 750_b, OD647_b, and OD 630_b are of interest and should be recorded respectively.

7.3.6 If the OD 664_b is > 0.045 then acidification is required.

7.3.6.1 Add 100 ul of 0.1 N H Cl to cuvette containing sample and mix gently. Start timer. After 90 seconds press the **Run** key. Read and record OD 665_a and OD 750_a .

7.3.7 Discard sample and repeat sections 7.3.3 to 7.3.6 for all samples.

8.0 Quality Control

8.1 The OD 664_b should be between 0.1 and 1.0. Do a dilution if necessary. For very dilute extracts use cuvetts having a longer path length, if available. If a larger cell is used, add a proportionally larger volume of acid.

8.2 The OD 750 reading is a correction for turbidity and should be less then 0.0200, if it is > 0.0200 then steps should be taken to correct the turbidity.

8.3 Samples with an OD 664_b/ OD 665_a ratio of 1.70 are considered to contain no pheophytin a and to be in excellent physiological condition.

8.4 The volumes of extract, of acid, time after acidification, and acetone-to-water proportions are critical for accurate, consistent results.

9.0 Data Analysis

9.1 The OD 750 nm turbidity reading must be subtracted from the other wavelengths before using them in the following equations.

9.2 Using the corrected values, calculate chlorophyll A and phenophytin A in ug/L as follows:

$$\text{Chlorophyll A, ug/L} = \frac{26.7(664b-665a) \times V1}{V2 \times L}$$

$$\text{Phenophytin A, ug/L} = \frac{26.7[1.7(665a)-664b] \times V1}{V2 \times L}$$

where:

V1 = volume of extract, ml
V2 = volume of sample (L)
L = light path length or width of cuvette in cm, and
664b, 665a = corrected optical densities of 90% acetone extract

9.3 Calculations for the determination of chlorophyll a, b, and c (trichromatic method) are included on the excel spread sheet but are not included in this SOP. The results reported out are corrected for phenophytin A.